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医薬品 研究報告 調査報告書

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販売名(企業名)	赤血球M・A・P「日赤」(日本赤十字社) 照射赤血球M・A・P「日赤」(日本赤十字社) 赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)					

研究報告の概要	使用上の注意記載状況・その他参考事項等
○シャーガス病が輸血用血液を通じて米国やヨーロッパに拡大—WHO WHOによると、感染の数十年後に死亡する可能性もある寄生虫症、シャーガス病が、不適切な血液スクリーニングが原因でラテンアメリカから米国やヨーロッパに拡大している。 WHOはバイエル社の支援を受けて、今や「地球規模の問題」となったシャーガス病根絶のための事業を拡大している。バイエル社は250万錠のNifurtimox(販売名:Lampit)を寄贈した。これは、若者の急性症例を含め今後5年間に3万人の患者を治療できる量である。 シャーガス病に感染している人は900万人にのぼると見られ、その多くはラテンアメリカの農村部の子どもである。最近では大規模な移民の影響で米国、スペインや他の欧州諸国に広がっている。シャーガス病は感染者に臓器の腫脹を引き起こし、最終的には死亡に至る病気で、正確な死亡率は不明である。大多数の感染者は、寄生虫を媒介する大型のナンキンムシに似た吸血昆虫に噛まれた後、感染していることを知らないまま数十年の潜伏期間を過ごすことになる。 「この病気はラテンアメリカの多くの人にとっては今でも脅威である。感染した供血者の適切なスクリーニングが行われていないため、血液銀行を通して脅威は他の国々に広がっている」とWHOの南北アメリカ担当者は話している。ジュネーブのWHO本部は、シャーガス病の撲滅に力を入れており、感染者数は1990年の1600～1800万人から減少してきた。 チリ、ウルグアイ、ブラジルの大部分、中米・アルゼンチン・ボリビア・パラグアイの広範囲の地域では、感染伝播は減少している。最も流行している地域は、ボリビアとアルゼンチンのチャコ地方、メキシコの一部、ペルー、コロンビアである。	赤血球M・A・P「日赤」 照射赤血球M・A・P「日赤」 赤血球濃厚液-LR「日赤」 照射赤血球濃厚液-LR「日赤」 血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク

報告企業の意見	今後の対応
シャーガス病が、ラテンアメリカから移住した人の供血を通して米国やヨーロッパに拡大しているとの報告である。	日本赤十字社は、輸血感染症対策として献血時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、シャーガス病の既往がある場合には献血不適としている。今後も引き続き情報の収集に努める。

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MedDRA/J Ver.10.0J

ıagas spread to U.S., Europe via blood banks -WHO
Apr 2007 13:47:09 GMT
urce: Reuters

NEVA, April 13 (Reuters) - Chagas, a parasitic disease which can kill victims decades after infection, has spread from Latin America to the ited States and Europe due to inadequate blood screening, the World Health Organisation said on Friday.

: United Nations agency said it was expanding its programme to eliminate Chagas, which has become a "global problem", with the help of /er HealthCare <BAYG.DE>.

/er's donation of 2.5 million tablets of Lampit, known generically as nifurtimox, will help treat an estimated 30,000 patients over the next five rs, covering new acute cases among youngsters, it said.

ıgas, which currently affects an estimated nine million people, mainly children in rural areas of Latin America, has emerged in the United tes, Spain and several other European countries after large-scale migrations, the WHO said.

exact death toll exists for the "silent killer" which causes the slow swelling of victims' internal organs, resulting in their eventual death, ording to the WHO.

st victims may not know they have contracted Chagas as the infection may remain dormant for decades after they have been bitten by a ɔd-sucking insect similar to a large bed bug which transmits the parasite.

ıis disease still poses a threat to so many people in Latin America and now that threat has spread to other countries via blood banks lacking quate screening of infected donors," said Mirta Roses Periago, WHO director for the Americas region.

: Geneva-based WHO has been working to wipe out the disease and the number of those infected has fallen from 16-18 million people in 0.

nsmission of the disease has been interrupted in Chile, Uruguay, a large part of Brazil, as well as vast areas of Central America, Argentina, ivia and Paraguay, the WHO said.

: most endemic regions remain the Chaco regions of Bolivia and Argentina, as well as parts of Mexico, Peru and Colombia, according to ıin.

REUTERS

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医薬品
医薬部外品　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2007年5月7日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	人ハプトグロビン		研究報告の公表状況	Transfusion 2007; 47(5): 883-889	公表国 アメリカ	
販売名（企業名）	ハプトグロビン注－ヨシトミ(ベネシス)					

研究報告の概要

＜背景＞
特に凝固因子でのパルボウイルスB19（B19）は一般的な汚染物質である。1999年にSD処理を施したプール血漿によるB19伝播を理由として、幾つかの分画メーカーは製造プールのB19負荷を制限するためにミニプールNATを開始した。本研究では、B19 NATスクリーニングが実施された前と後で製造された市販の第Ⅷ因子（AHF）製剤中のB19 DNA汚染の程度を確認した。
＜研究デザイン及び方法＞
1993-1998年及び2001-2004年の間に製造された6つのAHF製剤を代表する全部で284ロットを、in-houseのNAT法によりB19 DNAを測定した。抗B19抗体（IgG）も併せて測定した。
＜結果＞
1993-1998年に製造されたほとんどのロットからB19 DNAが検出された。陽性率は56～100％で、製造業者により異なっていた。検出されたB19 DNAの最高濃度は10^6 IU/mLであった。検査された40％のロットは10^3 IU/mLであった。対照的に、2001-2004年の間に製造された原料血漿由来のAHF製剤の陽性率及び濃度は低かった。しかしながら、回収血漿由来の製剤では変化が見られず、これはミニプールNATが実施されていなかったためである。中間的な精製度のAHF製剤のみが、抗B19抗体陽性であった。
＜結論＞
B19 NATをスクリーニングしていない血漿から調製したAHFのB19 DNAの陽性率及びレベルは高かったが、製造方法が異なると、製剤間でさまざまであった。血漿中のB19 NATスクリーニングは、最終製剤中のB19 DNAレベルを下げて大半の例で検出限界以下とさせ、このことでB19伝播のリスクを減少させた可能性がある。

使用上の注意記載状況・その他参考事項等

1. 慎重投与
(4) 溶血性・失血性貧血の患者〔ヒトパルボウイルスB19の感染を起こす可能性を否定できない。感染した場合には、発熱と急激な貧血を伴う重篤な全身症状を起こすことがある。〕
(5) 免疫不全患者・免疫抑制状態の患者〔ヒトパルボウイルスB19の感染を起こす可能性を否定できない。感染した場合には、持続性の貧血を起こすことがある。〕
2. 重要な基本的注意
(1)略
1) 血漿分画製剤の現在の製造工程では、ヒトパルボウイルスB19等のウイルスを完全に不活化・除去することが困難であるため、本剤の投与によりその感染の可能性を否定できないので、投与後の経過を十分に観察すること。
5. 妊婦、産婦、授乳婦等への投与
妊婦又は妊娠している可能性のある婦人には、治療上の有益性が危険性を上回ると判断される場合にのみ投与すること。〔妊娠中の投与に関する安全性は確立していない。本剤の投与によりヒトパルボウイルスB19の感染の可能性を否定できない。感染した場合には胎児への障害（流産、胎児水腫、胎児死亡）が起こる可能性がある。〕

報告企業の意見	今後の対応
原料血漿におけるミニプールNATの実施は、最終製剤中のB19 DNAレベルを下げ、B19伝播のリスクを減少させる可能性があるとの報告である。 本ウイルスは血漿分画製剤の製造工程での不活化・除去が困難であり、その伝播リスクを完全に否定できないため、1996年11月より、使用上の注意に本ウイルスの伝播リスクについての記載を行い、注意喚起を図っている。 また、原料への本ウイルス混入量低減のため、RHA(Receptor-mediated Hemagglutination)法を用いたドナースクリーニングによる高力価血漿の排除を行なっている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

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Parvovirus B19 DNA in Factor VIII concentrates: effects of manufacturing procedures and B19 screening by nucleic acid testing

Yansheng Geng, Chuan-ging Wu, Siba P. Bhattacharyya, De Tan, Zheng-Ping Guo, and Mei-ying W. Yu

BACKGROUND: Parvovirus B19 (B19) is a common contaminant, especially in coagulation factors. Because of B19 transmission by pooled plasma, solvent/detergent treated in 1999, some fractionators initiated minipool nucleic acid testing (NAT) to limit the B19 load in manufacturing pools. In this study, the extent of B19 DNA contamination in commercial Factor VIII concentrates, that is, antihemophilic factor (human) (AHF), manufactured before and after B19 NAT screening was implemented, was determined.

STUDY DESIGN AND METHODS: A total of 284 lots representing six AHF products made during 1993 to 1998 and 2001 to 2004 were assayed for B19 DNA by an in-house NAT procedure. Anti-B19 immunoglobulin G (IgG) was also measured.

RESULTS: Most lots made during 1993 to 1998 had detectable B19 DNA. The prevalence ranged from 56 to 100 percent and appeared to differ between manufacturers. The highest level of B19 DNA found was 10^6 genome equivalents (geq or international units [IU]) per mL. Forty percent of the lots tested contained 10^3 geq (IU) per mL. In comparison, both prevalence and levels in source plasma–derived AHF products made in 2001 to 2004 were lower. Both, however, remained unchanged in the recovered plasma-derived product because B19 NAT screening had not been implemented. Only an intermediate-purity AHF product was positive for the presence of anti-B19 IgG.

CONCLUSION: The prevalence and levels of B19 DNA in AHF prepared from B19 NAT unscreened plasma were high but varied among products with different manufacturing procedures. B19 NAT screening of plasma effectively lowered the B19 DNA level in the final products and in the majority of cases rendered it undetectable and hence potentially reduced the risk of B19 transmission.

Parvovirus B19 (B19) is a small nonenveloped DNA virus, known to resist viral inactivation procedures commonly used in manufacturing of plasma derivatives; it is widespread among populations.[1] The prevalence of B19 viremia in blood and plasma donors has been reported to range from 0.003 to 0.6 percent, depending on the time of an epidemic or the sensitivity of nucleic acid testing (NAT) methods.[2-4] Extremely high viremic levels in plasma, for example, 10^{13} genome equivalents (geq) of B19 DNA per mL, are often found at an early phase of the infection in acutely infected but asymptomatic donors.[5] As a consequence, B19 DNA has been detected at high frequency and high levels in plasma pools and their resulting plasma derivatives, especially the coagulation products.[6-8] Reports of transmissions attributed to Factor (F)VIII concentrates

ABBREVIATIONS: AHF = antihemophilic factor (human); B19 = parvovirus B19; VI/R = viral inactivation/removal.

From the Division of Hematology, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, Maryland.

Address reprint requests to: Mei-ying W. Yu, PhD, Division of Hematology, HFM-345, Center for Biologics Evaluation and Research, Food and Drug Administration, Room 303, 29 Lincoln Drive, Bethesda, MD 20892; e-mail: mei-ying.yu@fda.hhs.gov.

The findings and conclusions in this article have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy.

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(antihemophilic factor (human) [AHF]), subjected to solvent/detergent (S/D) treatment, heat treatment, or both, are numerous.[9-15]

Because of the B19 transmission in 1999 associated with pooled plasma, S/D-treated in a postmarket surveillance study that correlated product infectivity with a high concentration of virus in the manufacturing pool, plasma screening of B19 DNA by NAT in a minipool format was implemented as an in-process control test so that the viral load in the plasma pool used to manufacture the product could be limited to less than 10^4 geq per mL of B19 DNA.[16-19] The FDA has since proposed a similar limit for manufacturing pools destined for all plasma derivatives to reduce the potential risk of transmission.[19-23] Beginning in late 1999, some fractionators, mostly those who use source plasma, initiated (albeit gradually) the use of less sensitive, or so-called high-titer, minipool NAT screening to lower the viral load in manufacturing pools.[4,20-24] Some final products obtained from minipool-screened plasma have been found to be devoid of B19 DNA contamination.[4] The sensitivity of these minipool NAT tests varied but, in general, they excluded donations with B19 DNA levels of 10^6 geq per mL. In 2001, the Plasma Protein Therapeutics Association issued voluntary standards calling for manufacturers to implement 1) minipool screening of incoming plasma no later than the end of 2001 and 2) manufacturing pool testing to achieve levels of B19 DNA not to exceed 10^5 IU per mL no later than July 1, 2002.[23] Since then, all source plasma and manufacturing pools prepared from it have undergone B19 DNA testing.

The aim of this study was to evaluate the effect of B19 NAT screening of plasma on the resulting high-risk final products by comparing the prevalence and levels of B19 DNA in each of six US-licensed FVIII products made in two periods, that is, during 1993 to 1998 (before B19 NAT screening was implemented) and 2001 to 2004 (when such screening was nearly universal). Because the purification and viral inactivation/removal (VI/R) procedures used in the manufacturing of these products underwent little or no change over this entire span, the effectiveness of the B19 NAT screening could be evaluated, as could that of individual manufacturing procedures employed before any B19 screening.

MATERIALS AND METHODS

AHF samples

Six commercial AHF products represented by 136 lots made by five manufacturers during 1993 to 1998 and 148 lots made during 2001 to 2004, which were submitted by manufacturers to the FDA for lot release, were available for testing. The freeze-dried AHF products were reconstituted according to manufacturers' instructions, mostly with half of the specified volume, except that some lots made in 2004 were reconstituted with a full volume of the diluent. Unused reconstituted samples were stored at −70°C until further use.

DNA extraction and quantitation of B19 DNA by NAT

The extraction and semiquantitative NAT procedures were essentially the same as those described previously[15] except that a larger aliquot of reconstituted AHF, that is, 1.0 mL, was used for DNA extraction. For sample extraction and B19 NAT, either the WHO International Standard (NIBSC 99/800, 10^6 IU of B19 DNA/mL when reconstituted) or the CBER standard for B19 DNA (10^6 IU/mL) was used as a control.[25] Both were diluted 10^3-fold before use. Briefly, DNA from each sample or standard was extracted by use of an isolation kit and procedures (NucliSens, Organon Teknika, Durham, NC), and the DNA was recovered with 100 μL of the elution buffer. Aliquots of 25 μL of the undiluted or $10^{0.5}$-fold serially diluted DNA extracts in duplicate were used to perform nested polymerase chain reaction with primers derived from the VP1/VP2 region. Levels (in geq/mL) of B19 DNA in samples were determined by limiting dilution analysis. The sensitivity of the NAT assay for the large-volume extraction is 4 geq per mL, and the conversion ratio from geq to IU is 1:1.[15] This B19 NAT procedure detects both Genotype 1 and Genotype 2 of B19 but not the Genotype 3 variant (see Discussion).

Detection of anti-B19

Anti-B19 immunoglobulin G (IgG) was detected by use of a B19 IgG enzyme immunoassay kit (Biotrin International Ltd, Dublin, Ireland) according to the manufacturer's instructions except that a large sample aliquot, that is, 100 μL, was used for testing each reconstituted AHF. Most of the AHF samples tested were B19 DNA–positive.

Statistical analysis

The chi-square test was used to compare the prevalence between products. In addition, for comparing viral levels expressed as the log geometrical mean ± standard error of log geometrical mean (SEM), statistical analysis was performed by use of the unpaired t test. Results having p values of less than 0.05 were considered significant.

RESULTS

Prevalence and levels of B19 DNA in AHF lots manufactured during 1993 to 1998: effects of manufacturing procedures

Most products were made mainly from source plasma, but Product C was made from recovered plasma. The various purification and VI/R procedures used in the manufactur-

TABLE 1. B19 DNA prevalence and levels in FVIII concentrates made during 1993 to 1998*

Product	VI/R and purification	Positive lots/lots tested (%)	B19 DNA (geq/mL) among positive lots†						
			10^6	$\geq 10^5$	$\geq 10^4$	$\geq 10^3$	$\geq 10^2$	≥ 10	Log geomean ± SEM
A	S/D, affinity, dry/80°C/72 hr	9/13 (69)	0	0	2	2	2	3	2.3 ± 0.41
B	S/D, immunoaffinity (SP)	22/25 (88)‡	0	0	7	10	3	2	3.0 ± 0.20§
C	S/D, immunoaffinity (RP)	14/15 (93)‡	0	2	2	8	2	0	3.3 ± 0.24§
D	S/D, gel filtration chromatography	15/15 (100)‡	2	6	1	2	3	1	3.9 ± 0.42§
E	Wet/60°C/10 hr	16/25 (64)	2	2	2	1	5	4	2.9 ± 0.45§
F	Wet/60°C/10 hr, immunoaffinity	24/43 (56)	0	0	0	3	11	10	1.7 ± 0.14
Total		100/136 (74)	4	10	14	26	26	20	

* Product C from recovered plasma (RP) and all other products from source plasma (SP); information regarding viral inactivation/removal (VI/R) and product purification procedures are from products' package inserts.

† B19 DNA–positive lots were categorized into groups, according to the level of B19 DNA. For 1993 to 1998, no positive lot was found to have fewer than 10 geq per mL; hence this category was not used. Categorization was done according to the following example: the at least 10^2 group indicates the number of positive lots containing less than 10^3 but at least 10^2 geq per mL. Levels in the last column are expressed as log geomean ± standard error of log geomean (SEM) among positive lots.

‡ $p < 0.01$ when compared to Product F; $p < 0.05$ when compared to Product E.

§ $p < 0.01$ when Product B, C, or D compared to Product F; $p < 0.05$ when Product E compared to Product F.

ing process are indicated in Table 1. Detectable B19 DNA in these products ranged from 56 to 100 percent of the lots tested (Table 1). The prevalence in either Product F or Product E was significantly lower than that in Products B, C, and D. Product F was a high-purity AHF product subjected to both wet heating at 60°C for 10 hours and purification by immunoaffinity chromatography utilizing monoclonal antibody (MoAb) to von Willebrand Factor (VWF), whereas Product E was an intermediate-purity product subjected only to wet heating. Interestingly, both Product B and Product C, which were S/D-treated, high-purity AHF products subjected to purification by immunoaffinity chromatography utilizing MoAb to FVIII, had a significantly higher positive rate than the wet-heated, high-purity Product F. In addition, the prevalences in lots of Products B and C, which were derived from source plasma and recovered plasma, respectively, were 88 and 93 percent, and hence there appeared to be no difference in prevalence of B19 as a function of the type of starting plasma. Three of the products, viz., A, E, and F, underwent manufacturing that included a heating step. When these were compared with the other products (B, C, and D), the prevalence was found to be significantly lower ($p < 0.001$), suggesting that heating was partially effective in eliminating detectable B19.

The highest level of B19 DNA in AHF products was 10^6 geq per mL found in 2 lots each of Products D and E (Table 1). Among 100 B19 DNA–positive lots, 54 lots (54% of the positive lots or 40% of the lots assayed) contained 10^3 geq per mL. Product F, which had the lowest prevalence of positive lots, also had the lowest B19 DNA levels among all products. The S/D-treated, immunoaffinity-purified Product B or Product C had significantly higher levels of B19 DNA when compared to Product F. There was no apparent difference in levels between Products B and C, which were derived from different types of starting plasma. Comparing the level of B19 DNA in heated product (A, E, and F) with that in unheated products (B, C, and D), suggested that heating was somewhat effective in lowering B19 contamination. Products E and F, however, differed significantly in B19 content ($p < 0.01$), despite the fact that both were heated for 10 hours at 60°C in solution during manufacture. Thus individual manufacturing steps (e.g., heating and immunoaffinity purification) can apparently be additive in their effects on B19.

Prevalence and levels of B19 DNA in lots manufactured during 2001 to 2004: effects of B19 NAT screening

To investigate the effect of minipool NAT screening by B19 NAT, we assayed AHF products made during 2001 to 2004 and compared the results with those from corresponding product lots made during 1993 to 1998. This appeared to be a valid comparison, inasmuch as the VI/R and product purification procedures for AHF products were essentially unchanged over this time span. The prevalence of B19 DNA in lots of products made from source plasma (i.e., all except Product C) ranged from 13 to 27 percent (Table 2). Moreover, all positive lots (25/129) made from source plasma contained less than 10^3 geq per mL, with 21 lots containing less than 10^2 geq per mL. Among 4 positive lots detected in Products A, B, and D, which had less than 10^3 but at least 10^2 geq per mL of B19 DNA, 3 lots were made in 2002, whereas 1 lot was made in 2004. In contrast, the prevalence in Product C, which was made from recovered plasma and had not been screened for B19 DNA, was 90 percent and the level of B19 DNA ranged as high as 10^4 geq per mL, with 9 of the 17 positive lots (53%) containing 10^3 geq per mL. Thus B19 NAT screening of plasma effectively lowered the B19 DNA level in all five products derived from source plasma, and in 81 percent of these 129 lots B19 DNA was undetectable. The product made

TABLE 2. B19 DNA prevalence and levels in FVIII concentrates made during 2001 to 2004

Product	Positive lots/lots tested (%)	B19 DNA (geq/mL) among positive lots*					
		$\geq 10^4$	$\geq 10^3$	$\geq 10^2$	≥ 10	<10	Log geomean ± SEM
A	8/30 (27)	0	0	2	1	5	1.8 ± 0.54
B	3/24 (13)	0	0	1	2	0	1.6 ± 0.43
C†	17/19 (90)‡	2	7	5	2	1	2.9 ± 0.25§
D¶	4/16 (25)	0	0	1	1	2	1.4 ± 0.55
E	6/28 (21)	0	0	0	3	3	0.86 ± 0.14
F	4/31 (13)	0	0	0	0	4	0.54 ± 0.04
Total (C excluded)	25/129 (19)	0	0	4	7	14	

* See Table 1.
† See Table 1.
‡ $p<0.01$ when compared to Product B.
§ $p<0.01$ when compared to Product B.
¶ Product subjected to additional dry heating at 80°C for 72 hours during this period.

TABLE 3. Anti-B19 IgG in FVIII concentrates*

Product	Positive lots/lots tested	
	1993-1998	2001-2004
A	0/4	0/5
B	1/11	0/5
C	0/9	0/4
D	0/3	0/4
E	10/10	8/8
F	0/13	0/4

* For Products B and C, lots (made in 2001-2004) tested for anti-B19 were not exclusively B19 DNA–positive.

from unscreened recovered plasma exhibited not only a prevalence that was virtually unchanged over the two time periods but also levels of B19 DNA that were not significantly decreased.

Anti-B19 IgG in FVIII concentrates

A small sampling of lots (most of which were B19 DNA–positive) from each product was analyzed for the presence of anti-B19 IgG. B19 IgG was detected in all lots of Product E, an intermediate-purity AHF product whose manufacturing involved no chromatographic or affinity purification step (Table 3). In contrast, all other products except 1 lot from Product B were found negative.

DISCUSSION

During the mid and late 1980s, manufacturing procedures for FVIII concentrates changed greatly because of the inclusion of steps designed to improve product purity and/or to achieve VI/R. Since the early 1990s, however, the product purification and VI/R procedures for AHF products have remained largely unchanged. In this study, the prevalence and levels of B19 DNA in 136 lots representing six AHF products, that is, three high-purity products (Products B, C, and F) and three intermediate-purity products (Products A, D, and E), made in 1993 to 1998, were evaluated. Products B and C were made by an identical manufacturing procedure, whereas all other products were made by different methods.

B19 DNA has been reported to be prevalent in AHF products with levels as high as 10^7 geq per mL.[6,8] In our study, it was also found in the majority (74%) of the AHF lots made during 1993 to 1998, with levels of B19 DNA up to 10^6 geq per mL. Moreover, we found that the prevalence of B19 DNA was 100 percent in early AHF lots made in the 1970s (data not shown). The manufacture of these lots involved no VI/R steps, and B19 DNA levels sometimes reached 10^8 geq per mL. Our data are consistent with numerous reported B19 transmissions by FVIII concentrates subjected to either S/D or heat treatment or both.[9-15]

The wet-heated, high-purity Product F had the lowest prevalence and levels of B19 DNA among six products tested. Product E underwent a similar wet-heat treatment and also exhibited a low prevalence. It contained high levels of B19 DNA, however, apparently because its manufacturing lacks the immunoaffinity purification procedure used for Product F. An even more effective removal procedure for B19 might be developed by further exploiting this immunoaffinity-chromatography step (utilizing anti-VWF). Interestingly, for Product B or C, the immunoaffinity-chromatography step utilizing anti-FVIII has been validated and found to remove 4 logs of a model virus for B19, yet the prevalence and levels remained relatively high compared to those of Product F.

Products A, E, and F, all of which underwent manufacturing that included a heating step, had a significantly lower prevalence and B19 DNA levels than did unheated Products B, C, and D, suggesting that heating was partially effective in eliminating detectable B19. Product A was subjected to S/D treatment and affinity purification plus dry heating in the final container at 80°C for 72 hours. B19 is known to withstand dry heating at high temperatures, however, and transmissions have been documented in recipients of such heated AHF products.[11,12] The possible role of affinity purification in removing and hence lower-

ing B19 DNA in Product A cannot be ruled out because this method is used in combination with the dry heating. In contrast, our data for wet-heated Products E and F are consistent with the recent findings[26,27] that B19 can be susceptible to inactivation when heated in certain liquid media.

We also attempted to evaluate the possible effect of the type of plasma used on the prevalence and level of B19 in the resulting AHF product. This came about because Products B and C were derived exclusively from source plasma and recovered plasma, respectively, but made by the same manufacturing procedure. To obtain a product lot of comparable size, more units of recovered plasma are needed when compared to source plasma because of the difference in the volume of an individual unit. The results, however, suggest that there was no apparent difference because a similar prevalence and level of B19 DNA in the resulting product were obtained.

The prevalence of B19 DNA in blood and plasma donors can vary widely,[2-4,28] probably reflecting whether the collections were done at the time of an epidemic. The degree of B19 viremia in positive donations can also span a wide range,[4,28] with lower B19 DNA levels usually found in anti-B19 IgG–positive donors.[28] Likewise, before the implementation of B19 NAT screening, levels of B19 DNA in manufacturing pools were reported to range widely,[3,4,6-8] reaching as high as 10^9 geq per mL. These variations can, possibly, give rise to fluctuations in the levels of B19 DNA in final products—such as those observed in this study. Nonetheless, the consistent statistical differences in prevalence and level of B19 (Table 1) led us to conclude that individual manufacturing steps may have a significant effect in clearing, that is, inactivating and/or removing, B19.

The introduction of B19 NAT screening of source plasma after 1999 afforded us an opportunity to evaluate the effect of the viral load in the starting plasma on both the prevalence and the level of B19 DNA in the final product. In AHF lots made in 1993 to 1998 (when B19 NAT screening was not yet implemented), a total of 40 percent of the 136 lots tested, or 54 percent of the B19 DNA–positive lots, contained 10^3 geq per mL. In contrast, in AHF lots made in 2001 to 2004, B19 NAT screening of plasma effectively lowered B19 DNA levels in all five products derived from source plasma so that, of the 129 lots tested, *none* had a level of 10^3 geq per mL, and in 81 percent of the lots tested B19 DNA was undetectable. Obviously B19 NAT screening had not yet been implemented during this period for the recovered plasma destined for Product C, since the results were similar to those obtained from unscreened plasma in the earlier period. That is, 47 percent of the 19 lots tested, or 53 percent of the 17 positive lots, contained 10^3 geq per mL of B19 DNA.

Detection of B19 DNA in the product does not necessarily equate with infectivity. The actual infectious level of B19 in products is likely to depend on the level of anti-B19 IgG that is copresent in the product, in addition to the recipient's immune status. Anti-B19 IgG have been considered to be neutralizing antibodies and appear to confer lasting protection.[1] Although low viremic levels were found in donors who seroconverted to anti-B19 IgG,[28] seropositive sera have been shown by an in vitro infectivity assay system to contain neutralizing antibodies to B19.[29] Other studies[2,30] involving the infusion of B19 DNA–positive blood products with B19 DNA up to 2×10^6 geq per mL also demonstrated that when anti-B19 IgG was present either in the recipients or in the products, there was no B19 infection. In a recent publication,[31] it was reported that two seropositive immunocompetent recipients with anti-B19 IgG levels of 19 and 39 IU per mL were not infected with B19 after receiving pooled plasma, S/D-treated containing high-titer B19 DNA (1.6×10^8 IU/mL) and anti-B19 IgG (72 IU/mL), whereas the seronegative recipients were infected. These studies have strongly suggested the protective role of anti-B19 IgG.

In our study, none of the products tested, with the exception of the intermediate-purity Product E and one lot of Product B, had detectable anti-B19 IgG. In view of the fact that at least 50 percent of adults have circulating anti-B19 IgG,[32,33] B19 antibodies will invariably be present in any large plasma pool. Interestingly, most manufacturing procedures for AHF must be effective in separating FVIII from IgG. A product containing B19 DNA but devoid of anti-B19 IgG would potentially be infectious in seronegative recipients.

The minimal infectious dose (in terms of B19 DNA) in seronegative individuals is unknown. In a recent case report, we found that infection occurred when a seronegative, immunocompetent patient with mild hemophilia received an S/D-treated, high-purity AHF product, which contained 10^3 geq per mL of B19 DNA and was devoid of any detectable anti-B19 IgG; the total dose infused was 2×10^4 geq.[15] This is the lowest infectious dose (in terms of B19 DNA) that has been reported for a product containing no anti-B19 IgG. In a separate transmission case, a seronegative child was also infected by infusing a dry-heat-treated FVIII concentrate, which contained 4×10^3 geq per mL B19 DNA, but the total dose in terms of B19 DNA was 4×10^6 geq, and the product's anti-B19 IgG content, if any, was unknown.[14] For pooled plasma, S/D-treated, which was anti-B19 IgG–positive, a higher dose of B19 DNA was needed to produce infection. Only those seronegative volunteers infused with a 200-mL dose of product lots containing more than 10^7 geq per mL B19 DNA were infected, whereas those infused with an equal volume of lots containing less than 10^4 geq per mL did not seroconvert.[16,17,19] It remains to be determined whether AHF products containing low residual levels of B19 still may transmit the infection to susceptible individuals (presumably seronegative persons), especially to those high-risk individuals who are immune deficient.

B19 virus isolates have recently been classified into three genotypes with the majority of the isolates grouped as Genotype 1.[34] Like Genotype 1, Genotype 2 DNA was detected in FVIII concentrates as a contaminant but with lesser prevalence (2.5%); interestingly all Genotype 2-positive lots were also Genotype 1-positive.[35] The infectivity of a B19 Genotype 2 virus was recently found to be similar to that of Genotype 1 in an in vitro assay.[36] Genotype 3 has not been reported in FVIII concentrates. The lack of such a report, however, may be due to the fact that some B19 NAT procedures detect only Genotype 1 but not variant Genotype 2 or 3.[37] The NAT procedure used in this study detects both Genotype 1 and Genotype 2 but does not detect Genotype 3.

In conclusion, we have demonstrated that the prevalence and levels of B19 in FVIII concentrates made from plasma that was not screened for B19 DNA were high but varied among products with different manufacturing procedures. Minipool NAT screening for B19 DNA effectively lowers the prevalence and level of B19 in AHF. The majority of the lots of AHF now being manufactured have no detectable B19 DNA, and thus the risk of B19 transmission may be greatly reduced.

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